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The Effect of Photocatalytic Titanium Dioxide and Ultraviolet B Radiation on Sclerotinia homoeocarpa Growth and Pathogenicity

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To the Graduate Council:

I am submitting herewith a thesis written by Chun Hu entitled "The Effect of Photocatalytic Titanium Dioxide and Ultraviolet B Radiation on Sclerotinia homoeocarpa Growth and Pathogenicity." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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The Effect of Photocatalytic Titanium Dioxide and Ultraviolet B Radiation on *Sclerotinia homoeocarpa* Growth and Pathogenicity

A Thesis Presented for the

Master of Science

Degree

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Chun Hu

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DEDICATION

I dedicate this Thesis to my parents, Mingdong and Dehua, who have provided with more love, encouragement and support any daughter could hope for. Also, to the love of my life, my husband Lucas Freshour, thank you for being around with me, caring about me and loving me. I am looking forward to the many years we have ahead of us.

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ABSTRACT

Photocatalytic TiO_2 [titanium dioxide] generates strong oxidative effects when illuminated with ultraviolet (UV) light with wavelengths of less than 385 nm. UVB has wavelengths ranging from 280 to 315nm. Previous research indicates the oxidative species and UVB radiation can react and cause cellular damage to microorganisms, which may reduce *Sclerotinia homoeocarpa* growth and help to control dollar spot disease development. The objectives of this study were to investigate the interactions of TiO_2 and UVB radiation, both *in vitro* and *in vivo,* on the growth and development of dollar spot. Factorial treatments consisting of five rates of TiO_2 and three doses of UVB radiation were arranged in a completely random design. The *in vitro* study showed that the mycelia linear extension of *S. homoeocarpa* was significantly inhibited by UV radiation. The highest UVB radiation (0.2754mol $m^{-2}day^{-1}$[mol per square meter per day]) resulted in the least mycelium growth. Regression analysis predicted that 14 days after inoculation the mycelium diameter of plates exposed to the highest UVB radiation without TiO_2 would be 27mm; while the non-treated control was predicted to be 243mm. There were no significant differences among TiO_2 rates compared to the non-treated control. Results were similar in the *in vivo* study where creeping bentgrass (*Agrostis stolonifera* L) was grown in pots in a growth chamber, and exposed to three doses of UVB radiation for 12 hours per day and 4 rates of TiO_2. The lowest UVB radiation (0.04549 mol $m^{-2}day^{-1}$) treatment resulted in the largest infected area (58mm in diameter). Medium and high UVB radiation doses (0.1064 mol $m^{-2}day^{-1}$and 0.5444 mol $m^{-2}day^{-1}$, respectively) reduced the infected area to 40mm and 46mm 14 days after inoculation respectively. Overall, it appears that UVB radiation may be involved in regulating the overall size of *S. homoeocarpa* growth, and applications photocatalytic TiO_2 may result in an increase in disease due to its UV screening capability.

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CHAPTER 1

LITERATURE REVIEW

DOLLAR SPOT

Dollar spot is a common name for a foliar disease of turfgrasses caused by *Sclerotinia homoeocarpa* F. T. Bennett (Bennett, 1937). It is the most economically important disease on high maintained turfgrasses in the United States. It costs more money to manage dollar spot than any other disease found on golf courses (Vargas, 2005). For example, a typical golf course may spend several thousand dollars per hectare greens per year to control the disease. The pathogen is widespread, and it has been found in Australia, Central America, Europe, Japan, New Zealand, North America, United Kingdom and South China (Couch 1995, Fenstermacher 1980, Vargas 2005, Lv et al., 2009).

Symptoms and Epidemiology

Symptoms of dollar spot on turfgrass vary depending on the host species and management methods used. Dollar spot appears as small, sunken patches of up to 50 mm in diameter on closely mowed grasses (such as golf course greens), which are initially brown and then turn a bleached straw color. On taller grasses (fairways, home lawns), patches of blighted grass can range from 60 to 120 mm in diameter. The dead patches may become numerous and coalesce into large, irregular shapes if the disease becomes severe (Smiley et al., 2005).

Small, yellow-green, chlorotic lesions with a reddish-brown border may be seen on infected leaves, and they generally enlarge to extend across the entire leaf. Lesions often appear hourglass-shaped, but on some warm- and cool- season grass species, lesions may be oblong or oval and separated from healthy tissue by a brown border. Die back from leaf tips is also common (Smiley et al., 2005). A white cobweb-like mycelium may be seen growing on the surface of turfgrass and extending from leaf to leaf in early mornings when dew is present

(Monteith et al., 1932; Smith, 1955). The aerial mycelia can often be confused with mycelia of *Pythium, Rhizoctonia* and *Nigrospora* pathogens (Smiley et al., 2005).

Dollar spot has a wide host range, including most warm- and cool-season turfgrass species, and it is especially active on closely mown turfs such as creeping bentgrass and bermudagrass putting greens. The disease most commonly occurs at temperatures of 15-30 ℃ when warm, humid days and cool nights favor dew formation. These weather conditions occur most often in the late spring, and then in early autumn. It is also possible to observe dollar spot at other times of year as long as the environmental conditions are conducive to disease development. Dollar spot is more severe under conditions of low soil moisture and nitrogen fertility (Smiley et al., 2005; Vargas, 1994). Recent research showed that a logistic regression can be used to develop a model to predict the probability of dollar spot development on creeping bentgrass using weather variables. A predictive model would be useful for timing fungicide applications to protect high-value turfgrass (Smith et al., 2010).

When the environment is unfavorable, *S. homoeocarpa* overwinters as dormant mycelium in infected grass tissues and crowns, and as stromata on leaf surfaces (Fenstermacher, 1980). It is long believed that *S. homoeocarpa* is spread by infected grass clippings and maintenance equipment, such as mowers, irrigation hoses, and golf cart tires. However, this theory has conflicted with other research showing that the spatial structure of dollar spot remains relatively unchanged regardless of disease severity, suggesting that the factor primarily responsible for the spatial pattern is one that does not move about in space (Horvath, 2007). There is no evidence that *Sclerotinia homoeocarpa* is a seed-borne or soil-borne pathogen (Agarwal, et al., 1997). But recent research suggests that up to 0.07% of turfgrass seed may be infested by *S. homoeocarpa* and this could represent a potential source of pathogen inoculum (Kerns et al., 2011). During

periods favorable for pathogen development, mycelium within previously infected tissue or from stromata directly penetrates into leaves. The infection can also occur through stomates, cut leaf tips and other mechanical wounds (Smiley et al., 2005). Bennett (1937) indicated *S. homoeocarpa* produces both conidia and sporophores for reproduction. Although apothecia in turfgrass swards were reported, sexual or asexual spores were believed to be of minor importance to the epidemiology of the disease. In North America the spores of *S. homoeocarpa* have not been detected (Carbone et al., 1993). Genetic analysis using vegetative compatibility and molecular data suggest that populations of *S. homoeocarpa* are clonal (Deng et al., 2002; Viji et al., 2004; DeVries et al., 2008), and seasonal dollar spot epidemics are caused by a single species in the northern United States (Powell et al., 2001).

Control and Management

Cultural practices are very important to effectively manage dollar spot. Avoiding long periods of leaf wetness is essential to prevent fungal disease development (Couch, 1973). Watering deeply and as infrequently as possible; not irrigating in the late afternoon or evening; and removing the dew by mowing and rolling in the early morning are common cultural methods for controlling dollar spot (Smiley et al., 2005). Water stress can also encourage dollar spot disease development. Couch (1960) found that turfgrass suffering from water stress had higher infection potential during dry seasons. Light, frequent nitrogen applications can reduce disease severity via faster removal of necrotic tissue, and enhanced recovery once infection has been arrested (Musser, 1950; Couch, 1995).

Cultural management alone is not enough when disease pressure is high. This makes fungicides necessary for high quality turfgrass. Both contact and penetrant fungicides are available for the control of dollar spot. Contact fungicides are immobile and affect only those

fungi present on plant surfaces. Their efficacy is limited by precipitation, irrigation and regular mowing and removal of clippings. Contact fungicides are more effective when applied as a preventive treatment rather than a curative treatment (Latin, 2011). Because of their multisite mode of action, no resistance to contact fungicides among *S. homoeocarpa* populations has been reported. Penetrant fungicides, also called systemic fungicides, applied to plant surfaces are absorbed into underlying tissues. Compared to contact fungicides, systemic fungicides are more effective for long-term disease control. Demethylation inhibitors (DMIs), benzimidazoles and dicarboximides are the commonly used classes of systemic fungicides for dollar spot control.

However, as a consequence of frequent fungicide applications, fungicide resistance of the dollar spot pathogen *S. homoeocarpa* has been a consistent problem for more than four decades (Smiley et al., 2005). Several fungicide groups have been reported from various regions throughout the United States that were resisted by *S. homoeocarpa*, including cadmium-based fungicides (Cole et al. 1968, Massie et al. 1968); benzimidazoles (Cole et al.1974, Detweiler et al. 1983, Goldberg & Cole 1973, Warren et al. 1974); anilazine (Nicholson et al. 1971); dicarboximides (Detweiler et al. 1983); and the demethylation inhibitors (DMIs) (Doney and Vincelli 1993, Golembiewski et al. 1995), Furthermore, the management of fungicide resistance in populations of *S. homoeocarpa* has been compounded by the development of multiple-resistance to fungicides in various families. Benzimidazole-resistant strains with resistance to cadmium (Warren et al. 1974, Warren et al. 1977) or dicarboximides (Detweiler et al. 1983) have been reported. Some DMI-resistant strains were found that were multi-resistant to benzimidazole and the dicarboximide fungicides (Golembiewski et al. 1995). In Tennessee and Northern Mississippi, half of the tested isolates from 14 locations exhibited resistance to dicarboximide and benzimidazole classes, and two isolates showed resistance to DMIs. Multi-resistance was

observed as well, including resistance to benzimidazole and dicarboximide, benzimidazole and DMIs, as well as resistance to all three classes (Baird, 2005). Genetic analysis using vegetative compatibility, conserved gene amplification and DNA sequencing conducted on fungicide-resistant *S. homoeocarpa* isolates from Tennessee and Northern Mississippi showed that fungicide resistance was not associated with a particular vegetative compatibility group. This result may be due to the genetic similarities of isolates. Lack of sexual recombination probably caused the low diversity of the isolates (DeVries, R. E., 2008). Thus, alternative methods to control dollar spot are needed as most fungicide programs are expensive and fungicide resistance develops rather readily.

As early as the early 19th century, Bordeaux mixture was used to control brown patch and dollar spot. As the first inorganic fungicide, Bordeaux mixture was a combination of copper sulfate and hydrated lime (Monteith et al., 1932). But Bordeaux mixture can be harmful to fish, livestock and even humans, as a result of the potential build-up of copper in the soil (Pears et al., 2005). It is rarely used anymore on turfgrasses. In the last two decades, alternative tools for the management of dollar spot have focused on biological control agents. *Trichoderma harzianum* strain T-22 (Marketed as Bio-Trek) was registered in 1996 with the U.S. Environmental Protection Agency as the first biological fungicide for the control of a fungal disease of turfgrass (Gardner et al., 1996). As a preventative method, strain T-22B can colonize the plants roots and parasitize other fungi, protecting the plants through competition and infection of the pathogen (Howell, 2003). But a chemical fungicide must be applied once the disease symptoms occurred. The first bacterial biofungicide for turf was *Pseudomonas aureofaciens* strain TX-1 (Powell et al., 2000; Latin, 2011). The bacterium produces an active metabolite named, phenazine carboxylic acid, which has demonstrated strong antifungal activity (Dwyer, 1999; Powell et al., 2000).

Bacillus licheniformis strain SB3086 is another natural soilborne bacterium whose metabolites have been shown to have antifungal properties, and this bacterium was registered as Ecoguard biofungicide (Drahos, 2004). To achieve adequate dollar spot control, commercialized fermentation and delivery systems are needed; high inoculum concentration and high application frequency are critical as well (Dwyer, 1999). These limitations often result in a high cost of application, and a general lack of control with biofungicides.

CREEPING BENTGRASS

Creeping bentgrass (*Agrostis stolonifera* L.) is a perennial, cool-season turfgrass that widely used on golf course tees, greens and fairways, due to its fine texture and adaptation to mowing heights as low as 3 mm. Creeping bentgrass was originally adapted to cool, humid regions, but it has also been used in warmer climatic areas because of the high quality putting surface it provides (Warnke, et al., 2003).

Creeping bentgrass is susceptible to many diseases, such as dollar spot, brown patch, *Pythium* blight, and snow mold. The severity of diseases affecting creeping bentgrass will vary among different regions with differing climatic conditions. For example, hot and humid summer conditions favor dollar spot in the upper Midwest, Eastern coast, and Southern regions of the United States. However, in the Southwestern states dollar spot is not a significant problem because of the low humidity (Warnke, et al., 2003).

Developing host plant resistance to dollar spot would be the most ideal way to control the disease and reduce the fungicide requirements. In recent years a number of creeping bentgrass cultivars have been released with improved characteristics, including increased drought tolerance, shoot density and dollar spot disease resistance (Beard, et al., 2001; Bonos, et al., 2006; Liu et al., 2001; Stier, et al., 2003). The final report of 2004 to 2007 of the National Turfgrass Evaluation

Program indicated that creeping bentgrass cultivars Declaration performed the best in the four years of the trial, showing the least incidence of dollar spot. Further, 13-M, Memorial (A03-EDI) and Pennlinks II also performed well (NTEP, 2008).

But the costs of a golf course renovation are extremely high and easily exceed normal chemical and fertilizer budgets. Recent research tested eight creeping bentgrass cultivars: Penncross, Declaration, Memorial, PennA-1, PennA-4, LS-44, Syn-96, and PennG-2. Only Declaration and Memorial showed greater resistance to the dollar spot pathogen relative to the other six cultivars tested. None of the cultivars tested suppressed dollar spot severity even with monthly applications of reduced-rate fungicides (Koch et al., 2013). These results suggested that choosing a cultivar for a putting green renovation based solely on resistance to dollar spot will not lead to a reduction in fungicide usage substantial enough to justify the cost of renovation (Koch et al., 2013).

TITANIUM DIOXIDE

Titanium dioxide (TiO_2) is an inexpensive, multiuse compound. Because it has no absorption in visible region of the electromagnetic spectrum (Frazer, 2001), it's the ingredient in white paint that produces the brilliant white color. Considered by the U.S. Food and Drug Administration (FDA) to be non-toxic, it is widely used in our food and cosmetics. It is chemically stable in dark conditions, but when exposed to UV radiation, it shows photocatalytic properties and induces some chemical reactions (Hashimoto et al., 2007). Anatase, rutile and brookite are the three different crystalline structures of TiO_2. Anatase and rutile are commonly used and studied because of the easy transformation from brookite to rutile (Diebold, 2002).

Mechanism of TiO_2 Photocatalysis

The photocatalytic properties of TiO_2 powders were discovered in the 1950s. TiO_2 powders were dispersed into various organic solvents such as hydrocarbons and alcohol, and exposed UV radiation using Hg lamps. It was observed that the TiO_2 caused autooxidation of the solvents and the simultaneous formation of H_2O_2 under ambient conditions. Researchers compared the photocatalytic activities of various TiO_2 powders using twelve types of commercial anatase and three types of rutile, and concluded that the anatase form resulted in much higher autooxidation than that observed by the rutile form (Hashimoto et al., 2007).

In 1972 Fujishima found when the surface of a TiO_2 electrode was irradiated with light consisting of wavelengths shorter than 415 nm; photocurrent flowed from the Pt counter electrode to the TiO_2 electrode through the external circuit. The direction of the current revealed that the oxidation reaction (oxygen evolution) occurs at the TiO_2 electrode and the reduction reaction (hydrogen evolution) at the Pt electrode. This observation shows that water can be decomposed, using TiO_2 as a photocatalyst with UV light to activate the reaction, and split water into oxygen and hydrogen, without the application of external voltage. These reactions summarize Fujishima's work confirmed in 1972:

$TiO_2 + h\nu \rightarrow e^- + h^+$ (At the TiO_2 electrode)

$2H_2O + 4h^+ \rightarrow O_2 + 4H^+$ (At the Pt electrode)

$2H^+ + 2e^- \rightarrow H_2$

The overall reaction is

$2H_2O + 4h\nu \rightarrow O_2 + 2H_2$

However, the H_2 production is not very high because TiO_2 can only absorb UV light from the solar spectrum, of which UV light constitutes about 3% of the total light in the solar spectrum.

As a result of this work, the use of photocatalyzed oxidation from TiO_2 attracted more attention. In 1977, Frank et al. (1977) reported the first use of TiO_2 to decompose pollutants where they showed decomposition of cyanide in the presence of aqueous TiO_2 suspensions. In the late 80s and early 90s, degradation of various harmful compounds in both water and air using powdered TiO_2 was demonstrated and actively explored as a potential purification method for wastewater and atmospheric pollution (Ollis, 1985; Behnke et al., 1987; Hidaka et al, 1992; Yamazaki et al., 1994; Ahmed et al. 1995; Malati 1995).

As mentioned earlier, H^+ generated in TiO_2 was highly oxidizing; the constitutive elements of harmful compounds were oxidized completely by H^+, to their final oxidation state. In addition, various forms of reactive oxygen species (ROS), such as O_2^-, OH, $HO_2 \cdot$and O ; produced by the following processes may be responsible for the decomposition reactions as well (Hashimoto, et al., 2007).

$e^- + O_2 \rightarrow O_2^-$

$O_2^- + H^+ \rightarrow HO_2 \cdot$

$h^+ + H_2O \rightarrow$ OH + H^+

$h^+ + O_2^- \rightarrow 2$ O

Use of TiO_2 Photocatalysis

The photocatalytic decomposition reaction of TiO_2 can be applied to sanitizing areas of microorganisms. For example, *Escherichia coli* (*E. coli*) cells are killed when placed on TiO_2 following one week of UV irradiation at 1 mW cm^{-2}intensity. Cell deactivation is achieved in a much shorter irradiation time of 1 hour under normal solar UV light intensity (Sunada et al., 1998). Further research has found that the reactive oxygen species (ROS) which produced by TiO_2 photocatalytic reaction, can cause the peroxidation of the polyunsaturated phospholipids,

thus inducing a major disorder in the *E. coli* cell membrane (Maness, 1999). Kuhn (2003) used both light and scanning electron microscopy to observe that the microbial damage occurs through direct damage to cell walls caused by the hydroxyl radical (OH ·).

As a result of the anti-microbial activity of TiO_2, it has been widely used as a self–cleaning and self–sterilizing material for coating many clinical tools including sanitaryware, food tableware and cookingware, and items for use in hospitals (Fujishima, 1999).

In the last decade, many researchers have observed that TiO_2 also has use as an antifungal compound. Research has shown that TiO_2 may reduce post-harvest rots of fruit, such as *Diaporthe actinidiae*, a major fungal pathogen of kiwifruit (*Actinidia deliciosa*) (Hur, 2005); and *Penicillium expansum*, one of the most important fungal postharvest rots in fruits and vegetables (Maneerat et al., 2005). Photocatalytic disinfection times were shorter and disinfection with $TiO_2$was better than solar-only disinfection (Sichel et al., 2005). TiO_2 photocatalysis also reduced the severity of *cercospora* leaf spot and brown blotch of cowpea (*Vigna unguiculata* Walp) in the field (Owolade et al., 2008). The research showed that pathogens in the presence of photocatalytic TiO_2 and UV radiation lost viability as a result of their exposure. Furthermore, Owolade et al. (2008) showed that TiO_2 may also function as a growth promoter as cowpea yield increased by 8.74-36.11% and 10.33-51.31% in both years for treatments containing TiO_2. In 2010, 'Liquid composition for promoting plant growth containing TiO_2 nanoparticles' was published as a United States patent application, and this application suggested that TiO_2 nanoparticles might also increase the amount of chlorophyll in rice (*Oryza sativa*) (Kwang-soo, 2010).

ULTRAVIOLET RADIATION

Ultraviolet radiation (UVR) is a form of electromagnetic energy originates from the sun. It has a wavelength shorter than that of visible light, but longer than X-rays. The wavelengths of UVR range from 10 nm to 400 nm. UVR is categorized into three wavelength classes based on the biological effects of the photons on organisms: UVA, UVB and UVC. UVC has the shortest wavelength less than 290 nm. It is the most energetic and potentially most damaging of the wavelengths to organisms. However, UVC wavelengths are completely screened by ozone and oxygen in the upper atmosphere resulting in almost none of this type of UVR from reaching the Earth's surface. UVA wavelengths, from 320 to 400 nm, contribute 95% of the energy that UVR reaches the Earth's surface. UVA plays a major role in skin aging and wrinkling, and research has shown it directly affects the epidermis morphology and metabolism (Pearse et al., 1986). To stimulate the photocatalytic properties of TiO_2, UVA and UVB spectrum are needed.

Ultraviolet B (UVB)

Less than 5% of the UVR energy reaching the Earth's surface is from UVB radiation. UVB wavelengths range from 315 to 280 nm, which are in the middle of UVA and UVC. Most UVB is absorbed by oxygen and ozone in the upper atmosphere. But as atmospheric ozone levels decrease, researchers have concentrated on the effects of increased UVB exposure on biological systems, especially plants and humans (Rozema et al., 1997; Van der Leun, 1993). UVB radiation can induce a wide range of responses in plants, including increased concentrations of protective UVB-absorbing pigments in leaves, and decreased rates of CO_2 assimilation and plant growth (Kostina et al., 2001 and Teramura and Sullivan, 1994; Abney, 2009). Plant shoot height, shoot weight, photochemical efficiency and the concentrations of shoot tissue carotenoid and chlorophyll pigments in bunching onions (*Allium fistulosum*) were shown to be affected by UVB

(Abney, 2009). UVB exposure can also trigger the antioxidant defense system and increase the concentration of reactive oxygen species (ROS) in plant systems. This defense system consists of low molecular weight antioxidants such as ascorbate, glutathione, α-tocopherol, and carotenoids, as well as several enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase, and ascorbate peroxidase (Alscher and Hess, 1993; Bowler et al., 1994; and Sarkar et al., 2011). There has been some research investigating the ability of cool-season grass to tolerate UVB stress. Recent research indicated that 5.56 W $m^{-2}$of UVB irradiation for a single day significantly decreased the chlorophyll pigmentation of four cool season turfgrasses (creeping bentgrass 'L-92' and 'Penncross', tall fescue Holub, and perennial ryegrass) compared to non-irradiated controls. The visible loss of quality was most severe on creeping bentgrass compared to the untreated control (Nangle et al., 2011). Bartley (2012) found similar results showing that after one week of exposure to supraoptimal UV and PAR light creeping bentgrass chlorophyll and carotenoid leaf pigment concentrations were significantly reduced.

Unlike plants and humans, morphogenesis and especially sporulation of many fungi are very sensitive to UVB radiation (Ensminger, 1993; Vakalounakis, 1986; Fourtouni et al., 1998). Even mild doses of UVB radiation can restrict growth and reproduction of fungi. Fourtouni (1998) investigated the effect of UVB radiation on the fungus *Alternaria solani*. They observed that 3.2 KJ $m^{-2}day^{-1}$of UVB radiation reduced the colony surface area of the fungus by 31%. Dry mass production was reduced and increased hyphal density was observed. Willocquet et al. (1996) demonstrated greater spore germination and increased mycelia growth of the grape powdery mildew pathogen *Uncinula necator* under UVB absorbing Plexiglas compared with areas that received unfiltered full sunlight conditions.

Mechanisms of UVB phototoxicity may due to the high energy photons carried by UVB that causes direct damage to DNA by forming cyclobutane pyrimidine dimers, which disrupt DNA structure and induce mutation (Pfeifer, 1997; Griffiths, 1998). Field studies indicate that the UVB component of solar radiation plays an important role in the natural regulation of blister blight disease (caused by *Exobasidium vexans*). When UVB radiation was reduced approximately 75%-85% by a UV- screening filter, both the number of translucent spots and the number of sporulating blisters was increased(Gunasekera, 1997). Another field study demonstrated that solar UVB exposure alters phyllosphere bacterial community composition in peanut (*Arachis hypogeae* L.), especially in nonpigmented isolates. Late in the season UVR tolerant bacteria became a prevalent phenotype, and this may due to UVR selection pressure resulting in only UVR tolerant bacteria surviving. Other factors such as humidity decreases and temperature increases may also affect community composition (Jacobs, 2001).

CHAPTER 2

EFFECT OF TITANIUM DIOXIDE PHOTOCATALYST AND UVB RADIATION ON *SCLEROTINIA HOMOEOCARPA*

INTRODUCTION

Sclerotinia homoeocarpa F. T. Bennett is the pathogen that causes the turfgrass foliar disease, dollar spot (Bennett, 1937). Dollar spot is the most economically important disease on highly maintained turfgrasses in the United States (Vargas, 2005). More money is spent trying to manage dollar spot than any other diseases on golf courses (Vargas, 2005). Its distribution is widespread, including Australia, Central America, Europe, Japan, New Zealand, North America, United Kingdom and South China (Couch 1995, Fenstermacher 1980, Vargas 2005, Lv et al., 2009); and it has wide host range, including most warm- and cool-season turfgrass species. The disease most commonly occurs at temperatures of 15 to 30 ℃ under warm, humid days and cool nights that favor dew formation. On creeping bentgrass putting greens symptoms appear as small, sunken patches up to 50 mm in diameter, which are initially brown and then turn a bleached straw color. A white cobweb-like mycelium may be seen in early mornings when dew is present (Smiley, 2005). *Sclerotinia homoeocarpa* overwinter as dormant mycelium in infected grass tissues and crowns (Fenstermacher, 1980). The pathogen is believed to be spread via infested grass clippings by mowers and human activities. But this theory conflicts with other research showing spatial aggregation of dollar spot foci remains stable throughout a growing season, suggesting that the factor primarily responsible for the spatial pattern is one that does not move about in space (Horvath, 2007). When weather is favorable, the mycelium starts growing and penetrates into grass leaves via stomates, cut leaf tips and other mechanical wounds (Monteith et al., 1932; Smith, 1955). Spores of *S. homoeocarpa* have not been observed in North America, and genetic analysis using vegetative compatibility and molecular data suggested the pathogen is clonal (Powell et al., 2001; DeVries et al., 2008).

Removing the dew by mowing and rolling and increasing nitrogen fertility are the common cultural methods for controlling dollar spot (Monteith et al., 1937; Nikolai, 2002). Cultural management is not enough when disease pressure is high, and this makes fungicides necessary for high quality turfgrass. Generally, contact fungicides and systemic fungicides such as: DMIs, benzimidazoles and dicarboximides control dollar spot effectively. But fungicide resistance in populations of *S. homoeocarpa* has been a persistent problem since the 1960s, especially on golf courses utilizing the frequent fungicide applications (Smiley et al., 2005). Fungicide resistance in *S. homoeocarpa* have been reported from various regions throughout the United States which includes cadmium-based fungicides (Cole et al. 1968, Massie et al. 1968); benzimidazoles (Cole et al.1974, Detweiler et al. 1983, Goldberg and Cole 1973, Warren et al. 1974); anilazine (Nicholson et al. 1971); dicarboximides (Detweiler et al. 1983); and the demethylation inhibitors (DMIs) (Doney and Vincelli 1993, Golembiewski et al. 1995). Multi-resistance to more than one fungicide classes was reported as well. In Tennessee and North Mississippi, multi-resistance to benzimidazoles and dicarboximides, benzimidazoles and DMIs were observed. One location even had multi-resistance to all three classes (Baird, 2005).

Creeping bentgrass (*Agrostis stolonifera* L.) is a perennial, cool-season turfgrass that is widely used on golf course tees, greens and fairways, due to its fine texture and adaptation to mowing heights as low as 3 mm. Creeping bentgrass is originally adapted to cool, humid regions, but it is used in warmer climates because of a higher quality putting surface than bermudagrass (Warnke, et al., 2003). Creeping bentgrass is susceptible to many diseases, such as dollar spot, brown patch (*Rhizoctonia solani*), pythium blight (*Pythium aphanidermatum*), and pink snow mold (*Microdochium nivale*). Recently some creeping bentgrass cultivars have been released with increased disease resistance. For instance, the final report of 2004 to 2007 of the National

Turfgrass Evaluation Program indicated that creeping bentgrass cultivars Declaration performed the best in the four years of the trial, showing the least incidence of dollar spot (NTEP 2008). However, the costs of a golf course renovation are extremely high and easily exceed normal chemical and fertilizer budgets. Koch et al. (2013) suggested that none of the cultivars tested suppressed dollar spot severity even with monthly applications of reduced-rate fungicides. Thus, alternative methods to control dollar spot are needed as most fungicide programs are expensive and disease resistance develops.

Titanium dioxide (TiO_2) is an economical multiuse compound. It is widely used in 'self-cleaning' products such as countertops, fabrics, etc; and considered by the FDA to be non-toxic, and is used in human food, drugs and cosmetics. In 1972, Akira Fujishima found the photocatalytic properties of TiO_2. He indicated that TiO_2 photocatalyst generates oxidative species when illuminated with UV light with wavelength of less than 415 nm. The oxidative species have strong oxidizing power and can react and cause cellular damage to microorganisms through direct damage to cell walls caused by hydroxyl radical ($OH\cdot$) (Kuhn et al., 2003). Numerous research projects have shown that the antifungal activity of the TiO_2 photocatalyst can reduce post-harvest rots of fruits (Maneerat et al., 2005), and cercospora leaf spot and brown blotch in the field (Owolade et al., 2008). The presence of the TiO_2 photocatalyst under solar radiation showed a positive effect on the loss of fungus viability. The photocatalytic disinfection times were shorter and disinfection better than for solar-only disinfection of agricultural pathogenic fungi (*Fusarium* species) (Sichel et al., 2007). In 2010, 'Liquid composition for promoting plant growth containing TiO_2 nanoparticles' was published as United States patent application. The patent suggested that TiO_2 nanoparticles improved the amount of chlorophyll in rice (*Oryza sativa*) (Kwang-soo, 2010).

Ultraviolet radiation (UVR) is a form of energy originally from the sun. It has a wavelength shorter than that of visible light, but longer than X-rays, ranging from 10 nm to 400 nm. The sun emits ultraviolet radiation in the UVA, UVB, and UVC bands. To stimulate the photocatlytic properties of TiO_2, UVA and UVB are needed. Ultraviolet B (UVB) has the wavelength range is from 315 to 280 nm. Previous research proved that morphogenesis and especially sporulation of many fungi are sensitive to UVB radiation (Ensminger, 1993; Vakalounakis, 1986; Fourtouni et al., 1998). UVB radiation significantly reduced the radial growth of the fungus *Alternaria solani.* It also had a negative effect on dry mass production and stimulated increased hyphal density (Fourtouni et al., 1998). Mechanisms of UVB phototoxicity may be due to the high energy UVB photons causing direct damage to DNA by forming cyclobutane pyrimidine dimmers, which disrupt DNA structure and introduce mutation (Pfeifer, 1997; Griffiths, 1998)

Considering the favorable human safety and environmental impact qualities of TiO_2, and the anti-fungal activity of TiO_2 and UVB radiation, both of them were used in this study to determine their ability to control *S. homoeocarpa* growth and dollar spot disease development. Specific aims of these studies were to: 1) investigate antifungal activity of the photocatalytic TiO_2 and UVB radiation against the dollar spot fungi *S. homoeocarpa* in *vitro* and *in vivo*; 2) determine if the photocatalytic TiO_2 and UVB radiation prevent the fungal growth; 3) predict the growth rates under different TiO_2 and UVB radiation treatments; and 4) evaluate the dose response of TiO_2 and UVB radiation to restrict dollar spot growth.

MATERIALS AND METHODS

In Vitro

Sclerotinia homoeocarpa was isolated from diseased creeping bentgrass leaves from a bentgrass putting green at the East Tennessee Research and Education Center (Knoxville, TN). A 4mm sample was cut from the diseased leaf, cultured on ½ strength potato dextrose agar (PDA) plate at 25℃ for 7 days. After the white mycelium growth was visible, a 4mm agar plug containing fungal mycelia was cut and transferred to another ½ strength PDA plate, and cultured for 7 days to make the *S. homoeocarpa* fungal colonies.

Three types of TiO_2 material, Titanium Oxide (Anatase) in water dispersion, Titanium Oxide (Rutile) in water dispersion and Titanium Oxide (Anatase) nanopowder, were purchased commercially (Nanostructured & Amorphous Materials Inc, Houston, TX).

The ½ strength PDA plates with TiO_2 were made by mixing TiO_2 Anatase nanopowder with PDA powder and Bacto™ Agar powder (Becton, Dickinson and Company, Sparks, MD). Weights of 0g, 0.2g, 1g, 2g, and 10g TiO_2 Anatase nanopowder were added to 5 bottles separately. Each bottle received 3.9g PDA powder and 4g Bacto™ agar powder, and were filled to 200ml with distilled water to make ½ strength PDA solutions with 0 mg ml^{-1}, 1 mg ml^{-1}, 5 mg ml^{-1}, 10 mg ml^{-1} or 50 mg ml^{-1} TiO_2 respectively. All solutions were autoclaved under liquid cycle for 45 min in a 3043 Prevac Steam Sterilizer (STERIS Amsco Eagle, Mentor, OH), and then a 10ml solution was added to a 75mm petri dish using a motorized pipet (Fisher Scientific, Pittsburgh, PA). All plates remained in a laminar flow hood (NUAIRE S201-630, Plymouth, MN) until completely dry, and then stored at 4℃ for use as needed.

S. homoeocarpa plugs measuring 3.5mm were cut using a 3.5mm black sip stirrer (Royal, Coatesville, PA). Stirrers were sterilized before use using an autoclave as described previously.

Each fungal plug was placed on the center of a TiO_2 amended petri plate. Nine plates were used for each concentration of the TiO_2 amended media for a total of 45 inoculated plates.

The inoculated plates were either kept in dark or exposed to UV-B-313EL lamps (Q-Panel Lab Products, Westlake, OH) for 1 hr or 3 hr per day. All treatments lasted 7 days. Each plate represented one replication, three replications for each treatment. Plates were incubated at 25 ℃. The lamps were fixed at a constant height of 40 cm above the plates. Cumulative (mols $m^{-2} d^{-1}$) UVB radiation photon flux was measured using a UV sensor active in the wavelength ranges of 250-400 nm (Apogee Quantum19 and UVS Sensors, Apogee Instruments, Logan, UT). UVB radiation intensities of 0 mol $m^{-2} d^{-1}$; 0.0918 mol $m^{-2} d^{-1}$; and 0.2754 mol $m^{-2} d^{-1}$were applied in the 0 hr, 1 hr and 3 hr treatments, respectively. A daily cumulative UV radiation dose was also measured on a typical sunny day in September in East Tennessee Research Farm (Knoxville, TN), using the UV sensor.

Fungal growth was measured every 24 hr. Colony diameter was measured parallel to and perpendicular to an original mark made by a black pen, and the average diameter was calculated from these measurements.

The experiment was performed as a randomized complete block design with three levels of UVB radiation by five TiO_2 concentrations factorial and three replications. Data were analyzed through analysis of variance (ANOVA) using the MIXED procedure in SAS 9.3 (Statistical Analysis Software, Cary, N.C.), and least squares means compared using the Least Significant Difference method ($p=0.05$). Simple linear regression and response surface regression analysis were conducted, to predict the mycelium growth under different UVB radiation doses over time. Experimental repetitions were analyzed together as blocks due to a lack of a treatment by repetition interaction.

In vivo

Inoculum was prepared by culturing isolates of fungi on moist perennial ryegrass seed. 500 ml of perennial ryegrass seed was soaked in 750 ml of water for 24 hr, drained and autoclaved at 121 ℃ for 30 min twice in a 24 hr interval. An entire *S. homoeocarpa* fungal colony was cut from a PDA plate into 50-100 pieces, stirred into the ryegrass seeds, incubated at 25C for 2 weeks, and shaken well daily. The infested ryegrass seed was then used as inoculums for the experiment.

'Penn A-4' creeping bentgrass was seeded at 96 kg ha^{-1}into 10 cm diameter pots containing Fafard No.2 soil-less potting medium (Conrad Fafard, Inc., Agawam, MA, USA) and maintained in a greenhouse environment. Plants were initially fertilized every 7 days with a complete fertilizer (Vigoro All Purpose Plant Food 10-10-10, St. Louis, MO) at 48.8 kg N ha^{-1}. After 1 month the fertilizer was reduced to 24.4 kg N ha^{-1}per week. In order to maintain soil moisture, plants were watered daily with overhead irrigation. The creeping bentgrass plants were manually clipped with scissors twice weekly to maintain a height of approximately 1 cm.

After 2 months of growth in greenhouse all plants were inoculated with *S. homoeocarpa* using infested perennial ryegrass seed. Each creeping bentgrass pot received 10 infested seeds and was maintained in a mist chamber in the dark for 24 hr. Following the 24 hr period in the mist chamber, all pots were moved to a controlled environmental growth chamber (Conviron Adaptis A1000, Pembina, ND).

The growth chamber maintained a consistent temperature of 25 ℃ (day) and 15 ℃ (night) and plants were exposed to a 12 hr photoperiod. Three intensities of UVB radiation (0.04549 mol m^{-2} day^{-1}, 0.1064 mol m^{-2} day^{-1}, and 0.54435 mol m^{-2} day^{-1}) were applied using Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI). High

Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) were used as well to provide long wavelength visible light for plant photosynthesis. All creeping bentgrass pots were separated into three groups; each group was placed in a tray with standing water and a sealed lid to aid in pathogen development, and each tray received a single UVB radiation dose.

Four TiO_2 treatments of 0, 1, 10, and 50 mg TiO_2 ml^{-1} solution were made by diluting the 15% Titanium Oxide (Anatase) water dispersion with H_2O. Using a handheld pressurized sprayer (Preval, Precision Valve Corporation, Yonkers, NY) calibrated to deliver 4246 L ha^{-1}at 15 cm above the plant TiO_2 treatments were sprayed on creeping bentgrass canopies evenly. All treatments were applied on a 7-day interval immediately following a trimming, and the first application was sprayed 24 hr before inoculation.

Digital Image Analysis (DIA) (Richardson et al., 2001; Horvath, et al., 2005) was used to measure percent green cover. Digital images were captured every 3 days using a Canon G-12 digital camera (Canon U.S.A., Inc., Lake Success, NY) and an initial pretreatment image was made just before the initial TiO_2 treatments were applied. A light box with a red background was used to ensure that only the bentgrass canopy was captured in the images. Images were analyzed using Sigma Scan Pro © software (v. 5.0, SPSS, Inc., Chicago, IL). Percent green cover was evaluated. Percent disease infected area was calculated by comparing the initial green cover with the disease period green cover using the formula: percent diseased area = 100% - (disease period green cover/initial green cover) %. The diseased area was calculated by using percent diseased area * the pot area and the disease diameter can be calculated.

This study was a randomized complete block design with 3 by 4 factorial treatments (three intensities of UVB irradiation and four concentrations of TiO_2 treatment) with three replications. The entire experiment was repeated two times and marked as trial 1 and trial 2. The

two trails were analyzed together as blocks because there was not a significant treatment by trial interaction. . Data were analyzed through analysis of variance using the MIXED procedure in SAS 9.3 (Statistical Analysis Software, Cary, N.C.), and least squares means compared using the Least Significant Difference method (p=0.05).

RESULTS AND DISCUSSION

In vitro

There was no interaction among TiO_2 treatments and UVB radiation intensities across all times (Table 1). The differences of the fungal colonies under different UVB intensities tend to be the same regardless of the concentration of the TiO_2.

The pathogen was very sensitive to UVB radiation. Fungal growth was significantly different at various UVB intensities ($P<0.001$) over all times (Table 1). Higher UVB intensities resulted in smaller pathogen colonies (Table 3). Even without TiO_2 treatments, 3hr of UVB radiation per day at the doses supplied in this experiment restricted the pathogen diameter to an average of 1.87 cm 7 days after inoculation. Petri dishes kept in darkness achieved the maximum diameter of 8.5 cm 5 days after inoculation (Fig.1). The antimicrobial effect of UVB irradiation on several genera of fungi was observed by Fourtouni et al. (1998) and Willocquet et al. (1996). Fourtouni et al. (1998) found the same dose-dependent, inhibitory effect of UVB radiation on fungus *Alternaria solani*'s radial growth and dry mass production. But UVB radiation stimulated increased hyphal density, which was not observed in this study. Willocquet et al. (1996) found the mycelia growth of fungus *Uncinula necator* slowed significantly under both artificial UVB light and the sun exposure.

Total fungal growth was restricted by TiO_2 treatments for 2 days following inoculation (Table 1). The 50 mg ml^{-1} TiO_2 treatments resulted in significantly smaller fungal colony diameters (0.70; 1.38 cm) than those of the untreated control (1.04; 2.04 cm) (LSD=0.18; LSD=0.40) during the 2 days following inoculation (Table2). Treatments of TiO_2 without UVB radiation only affected *S. homoeocarpa* growth slightly. From Figure 2A, higher TiO_2 concentrations tended to have slower growth rates. Previous research (Matsunaga et al., 1985; Wet et al., 1994 and Maness et al., 1999) indicated that the disinfecting property of TiO_2 was positively correlated with an increasing dose of TiO_2. Maximum pathogen growth was delayed by 2 days in the 50 mg ml^{-1} TiO_2 treatment compared to the untreated control. Similar results were observed by Maneerat et al. (2006) where growth of *Penicillium expansum* was delayed 3 days when treated with TiO_2 only compared to an untreated control on tomato(*Solanum lycopersicum*) during storage. However, all tomato fruits showed fungal spoilage within 7 days after inoculation, except in the combination of TiO_2 and UV radiation. In the current study, TiO_2 alone did not inhibit the fungal growth significantly. No significant differences were observed among TiO_2 treatments under 1 hr of UVB radiation (Fig.2B). The 5 mg ml^{-1} TiO_2 treatment combined with a 3 hr UVB dose achieved the best result in our experiments, with the diameter of the pathogen reaching 0.97 cm 7 days after inoculation. However, this result was not significantly different from the UVB treatment without TiO_2 (1.87 cm) (LSD=1.3cm) (Fig. 1; Fig.2C). One possibility for this finding is that the high sensitivity of the pathogen to UVB radiation reduced the ability to detect the potential performance of the TiO_2 photocatalytic reaction. Additionally, we observed that TiO_2 concentrations greater than 5 mg ml^{-1} resulted in an increase in fungal growth (Fig.2C). Kim et al. (2003) has previously shown that when TiO_2 concentrations larger than 1 mg ml^{-1} are used, the ability of TiO_2 to kill bacteria is dramatically

reduced. This may be due to the fact that at such high concentrations the photocatalytic property of TiO_2 is also reduced. TiO_2 nanoparticles are effective skin-protective materials used in sunscreens that block UVB radiation (Popov, 2005; Innes et al., 2002). Popov (2005) found that the higher concentration of TiO_2 particles achieved better UVB incident radiation absorption. This work showed that UVB radiation decreased in the presence of TiO_2 from 23.5% to 13% (Popov et al., 2005). These results suggest that low concentration of TiO_2 may achieve better antifungal result, considered about its photocatalytic property and UVB absorption ability.

Regression analysis was conducted to examine the correlation between doses of UVB radiation and fungal growth over time to predict the colony surface area 14 days after inoculation. Only those data from the UVB radiation treatments without TiO_2 were used to conduct the regression. The regression model that fits the data predicts that fungal colony diameter will increase by 1.72, 1.16, and 0.2 cm per day for 0, 1, and 3 hr of UVB exposure, respectively (Table4). Three hr of UVB radiation significantly restricted fungal growth, and the predicted colony diameter after 14 days of exposure would be 2.74 cm. Ultraviolet B exposures of 0 and 1 hr would be predicted to achieve colony diameters of 24.39cm and 15.61cm respectively (Table 4). Dollar spot almost always appears as small circular patches that rarely exceed 5 cm in diameter in closely mowed grasses (Smiley et al., 2005). Our results suggest the possibility that UVB radiation could be involved in regulating the overall size of dollar spot symptoms. The sun is the Earth's primary source of UV radiation accounts for about 10% of the total energy that enters our atmosphere. Of the UV radiation that enters our atmosphere, approximate 95% is UVA and 5% is UVB (Oliva et al., 2005). According to the collected data, in East Tennessee, on a typical sunny day in September, the peak UV dose would be approximately 154 $\mu mol \cdot m^{-2} s^{-1}$ with a daily cumulative dose of 3.8 mol $m^{-2} d^{-1}$, and UVB radiation would be approximately

0.19 mol $m^{-2}d^{-1}$of the total cumulative dose. The response surface regression analysis predicts that the diameter of dollar spot symptoms with exposure to 0.19 mol $m^{-2}d^{-1}$of UVB radiation for 14 days would result in a diameter of 3.2 cm (Fig.3, Table 5). The fitted surface response curve was able to explain 90.45% of the variation (Fig. 3).

In vivo

Table6 presents significant differences among the TiO_2 treatments across all the timings, and difference among UVB radiation intensities 3 days and 14 days after inoculation. Similar to the *in vitro* experiment, there was no interaction among TiO_2 treatments and UVB radiation intensities across time.

The symptom development was significantly reduced by UVB radiation. Generally, our data show that the greater the UVB exposure the smaller diseased area (Table 8). Examining the pooled data across TiO_2 treatments, the diseased area was only 16.57% and 21.27% 14 days after inoculation under the two higher UVB exposures (0.5444 mol $m^{-2}day^{-1}$and 0.1064 mol $m^{-2}day^{-1}$) respectively. The lowest UVB exposure (0.1064 mol $m^{-2}day^{-1}$) increased the infected area to 33.46% (Fig.4). At the low level of UVB radiation, the diseased area was increasing at a much higher rate in comparison to medium and high UVB intensities (Fig. 4) 11 days after inoculation. The disease foci diameters were 40 mm and 46 mm 14 days after inoculation for medium and high UVB intensities respectively, and 58mm for the low UVB treatment which tended to continue to increase. Our results are similar to the observation by Smith (1955) where dollar spot colonies cultured in growth chamber conditions could grow much larger than they do in the field (up to 50 mm in diameter).

Significant differences were observed among TiO_2 treatments across the whole experiment (Table 6). However, unlike previous research, in our experiment disease severity was positively correlated with TiO_2 doses. In other words, the area of turf that was diseased increased with increasing TiO_2 dose. The highest TiO_2 concentration (50 ml mg^{-1}) had the largest diseased area at any time (Table 7; Fig.6). This result may be due to the high sensitivity *S. homoeocarpa* has demonstrated to UVB irradiation and the ability of TiO_2 nanoparticles to effectively screen UVB radiation (Popov, 2005; Innes et al., 2002). It is possible that when the pathogen penetrated into the grass leaves, the TiO_2 on the leaf surface was able to screen the UVB light, and thus reduce a factor that could restrict the pathogen development, and provide favorable conditions for the pathogen growth. It is also possible that the highest TiO_2 concentration treatment may have caused a phytotoxic reaction on creeping bentgrass. Chlorotic lesions were observed on leaf tips one day after the application of the 50 mg ml^{-1} TiO_2 treatment. Du et al., (2011) found that the wheat (*Triticum*) plants were harmed by TiO_2 nanoparticals, TiO_2 treatment had a decreased biomass production compared to control. They observed TiO_2 nanoparticals penetrated through the cell wall and accumulated on it, which may generate reactive oxygen species that could damage cell membranes. More research is needed to test this possibility on creeping bentgrass. No phytotoxicity was observed as a result of any UVB dose, but the UVB radiation doses in this experiment were relatively low compared to other studies (Bartley, 2012; Nangle et al., 2011).

CONCLUSION

This study revealed a potential relationship between the size of dollar spot foci and UVB radiation dose. It is possible that the dose of UVB radiation is a factor in why dollar spot does not typically develop foci larger than a silver dollar. This possibility needs to be tested in the

field using UVB screening Plexiglas to confirm the role UVB radiation plays in restricting the size of dollar spot foci. A better understanding of dollar spot biology could increase prediction accuracy of disease development, and more accurate predictions would allow improved timing of fungicide applications for disease control.

In our work, we showed that photocatalytic TiO_2 actually increases dollar spot symptoms and thus would not be recommended for use on creeping bentgrass. The highest TiO_2 treatment contained a large amount of TiO_2 nanoparticles that screen UVB radiation, and this may be the reason dollar spot was more severe in pots treated with the highest rate. Also, the reasons for the observed phytotoxicity caused by the highest TiO_2 treatment are still unclear.

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APPENDIX

A. TABLES

Table1. ANOVA table for *in vitro* TiO_2 treatment[1], UVB radiation[2], and their interactions for *Sclerotinia homoeocarpa* growth[3] 7 days after inoculation.

		Day						
Treatments	df	1	2	3	4	5	6	7
TiO_2	4	*	*	NS[4]	NS	NS	NS	NS
UVB	2	***	***	***	***	***	***	***
TiO_2*UVB	8	NS	NS	NS	NS	NS	NS	NS

*** Significant at the 0.05 level.**
**** Significant at the 0.01 level.**
***** Significant at the 0.001 level.**
[1] TiO_2 treatments included 5 doses of TiO_2: 0 mg ml^{-1}as untreated control, 1mg ml^{-1}; 5 mg ml^{-1}; 10 mg ml^{-1} and 50 mg ml^{-1}.
[2] Plates kept in dark (untreated control), or exposed to UVB fluorescent lights for 1 or 3 hours day^{-1}(0, 0.0918, 0.2754 mol $m^{-2}d^{-}$). UVB wavelengths are from 280 to 315nm.
[3] *Sclerotinia homoeocarpa* growth was determined by pathogen colony diameter (cm).
[4]Not significant at the $p<0.05$ level of significance.

Table2. Overall effect of TiO_2 concentration on *in vitro Sclerotinia homoeocarpa* growth[1] 7 days after inoculation.

TiO_2(mg ml^{-1})	Days after inoculation						
	1	2	3	4	5	6	7
	Pathogen colony growth (cm)						
0	1.04	2.04	3.06	4.12	4.90	5.48	5.98
1	0.85	1.67	2.53	3.64	5.00	6.00	6.37
5	0.88	1.70	2.58	3.61	4.80	5.56	5.88
10	0.86	1.60	2.71	3.89	5.25	6.23	6.64
50	0.70	1.38	2.33	3.37	4.62	5.72	6.10
LSD (0.05)[2]	0.18	0.40	0.78	1.11	1.38	1.64	1.68

[1] *Sclerotinia homoeocarpa* growth was determined by pathogen colony diameter (cm).
[2]Fisher's least significance difference value used to determine significant differences between means at $p<0.05$.

Table3. Overall effect of UVB irradiation[1] on *in vitro Sclerotinia homoeocarpa* growth[2] 7 days after inoculation.

UVB(Hour)	Days after inoculation						
	1	2	3	4	5	6	7
	Pathogen colony growth (cm)						
0	1.53	3.24	4.95	6.73	8.12	8.47	8.50
1	0.68	1.24	2.20	3.35	5.13	6.84	7.73
3	0.39	0.55	0.78	1.09	1.49	2.09	2.35
LSD (0.05)[3]	0.14	0.31	0.60	0.86	1.07	1.27	1.30

[1] Plates kept in dark (untreated control), or exposed to UVB fluorescent lights for 1 or 3 hours day^{-1}(0, 0.0918, 0.2754 mol m^{-2}d^{-1}). UVB wavelengths are from 280 to 315nm.
[2] *Sclerotinia homoeocarpa* growth was determined by pathogen colony diameter (cm).
[3]Fisher's least significance difference value used to determine significant differences between means at $p<0.05$.

Table4. Estimate of intercepts and slopes of the linear regressions between *Sclerotinia homoeocarpa* colony diameter (cm) and days after inoculation at each UVB radiation exposure duration[1] (equation: diameter=intercept + slope*day, p=0.05).

UVB(Hour)	Intercept(cm)	Slope(cm/day)	R-Square
0	0.31	1.72	0.97
1	-0.63	1.16	0.84
3	-0.06	0.2	0.42

[1] Plates kept in dark (untreated control), or exposed to UVB fluorescent lights for 1 or 3 hours day^{-1}(0, 0.0918, 0.2754 mol m^{-2}d^{-1}). UVB wavelengths are from 280 to 315nm.

Table5. Estimate of intercept and parameters for the response surface regression among pathogen colony diameter (y), days after inoculation(x_1), and UVB radiation exposure duration (x_2) (equation: $y=a+bx_1+cx_2+dx_1^2+ex_2^2+fx_1x_2$) (R-square=0.9045).

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.401285	0.336796	1.19	0.235
Day	1	1.797849	0.168058	10.7	<.0001
UVB	1	-1.192234	0.266315	-4.48	<.0001
Day*Day	1	-0.06261	0.020026	-3.13	0.0021
UVB*Day	1	-0.342616	0.02781	-12.32	<.0001
UVB*UVB	1	0.263492	0.074929	3.52	0.0006

Table6. ANOVA table for *in vivo* TiO_2 treatment[1], UVB radiation exposure[2], and their interaction for dollar spot disease development[3] on creeping bentgrass.

Treatments	df	0DPI[4]	3DPI	7DPI	11DPI	14DPI
TiO_2	3	NS[5]	***	***	*	*
UVB	2	NS	*	NS	NS	**
TiO_2*UVB	6	NS	NS	NS	NS	NS

*** Significant at the 0.05 level.**
**** Significant at the 0.01 level.**
***** Significant at the 0.001 level.**
[1] TiO_2 treatments included 4 doses of TiO_2: 0 mg ml^{-1}as untreated control, 1 mg ml^{-1}; 10 mg ml^{-1}; and 50 mg ml^{-1}.
[2] Creeping bentgrass pots inoculated with *Sclerotinia homoeocarpa* exposed to 3 doses of UVB lights, Low (0.0454 mol $m^{-2}d^{-1}$), Medium (0.1064 mol $m^{-2}d^{-1}$), and High (0.5444 mol $m^{-2}d^{-1}$).
[3]Disease development was determined by percent disease infected area using Digital Image Analysis (Richardson et al., 2001).
[4]DPI = days post inoculation.
[5]Not significant at $p<0.05$.

Table7. Overall effect of TiO_2 concentration on dollar spot disease development[1] on creeping bentgrass.

TiO_2(mg ml^{-1})	0DPI[2]	3DPI	7DPI	11DPI	14DPI
	Diseased area (%)				
0	0.00	8.07	11.00	19.03	23.77
1	0.00	8.62	12.23	22.54	28.41
10	0.00	10.19	16.52	24.86	31.10
50	0.00	16.08	29.83	36.20	46.10
LSD(0.05)[3]	0.00	2.77	7.53	9.37	12.48

[1]Disease development was determined by percent disease infected area using Digital Image Analysis (Richardson et al., 2001)
[2]DPI means day post inoculation.
[3]Fisher's least significance difference value used to determine differences between means at p<0.05 level.

Table8. Overall effect of UVB radiation[1] on dollar spot disease development[2] on creeping bentgrass.

UVB	0DPI[3]	3DPI	7DPI	11DPI	14DPI
	Diseased area (%)				
Low	0	10.87	20.58	30.30	43.32
Med	0	12.28	18.43	25.55	29.00
High	0	9.06	13.18	21.11	24.72
LSD(0.05)[4]	0	2.4	6.52	8.12	10.81

[1] Creeping bentgrass pots inoculated with *Sclerotinia homoeocarpa* exposed to 3 doses of UVB lights, Low (0.0454 mol $m^{-2}d^{-}$), Medium (0.1064 mol $m^{-2}d^{-}$), and High (0.5444 mol $m^{-2}d^{-}$). UVB wavelengths are from 280 to 315nm.
[2]Disease development was determined by percent disease infected area using Digital Image Analysis (Richardson et al., 2001)
[3]DPI means day post inoculation.
[4]Fisher's least significance difference value used to determine differences between means at $p<0.05$ level.

B. FIGURES

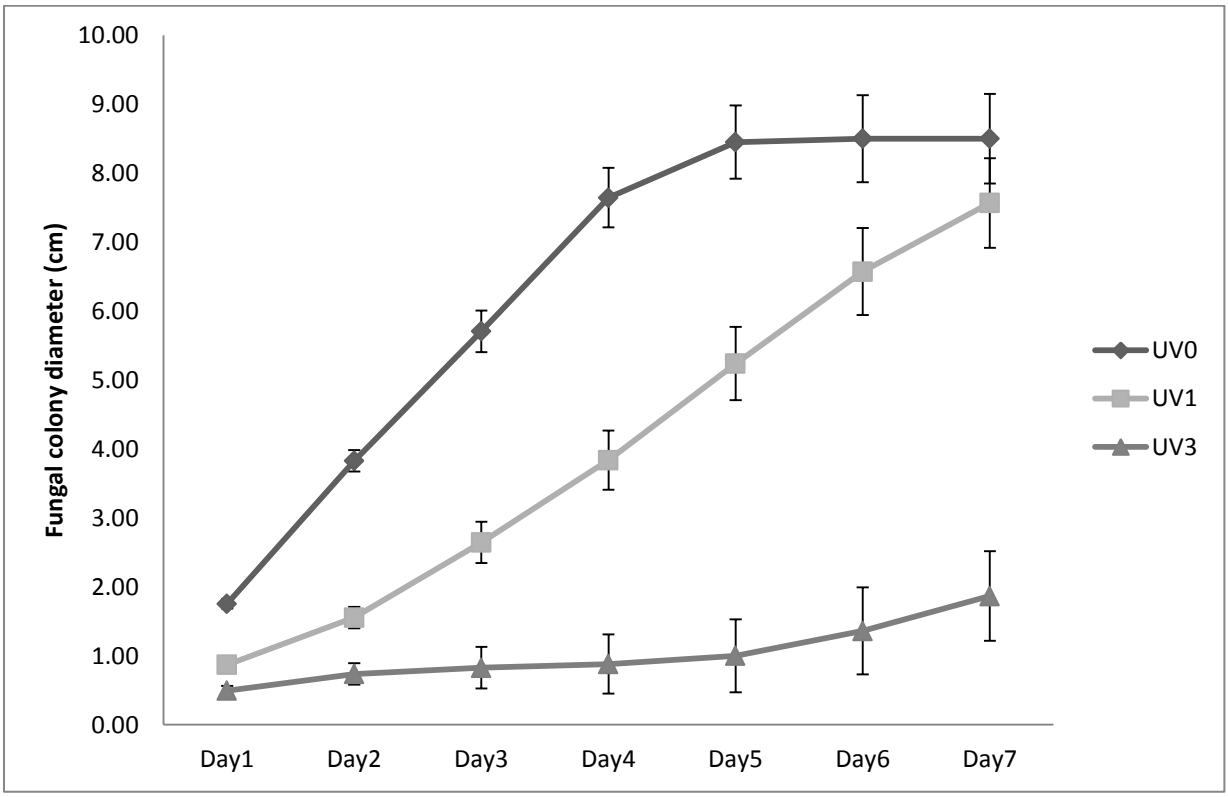
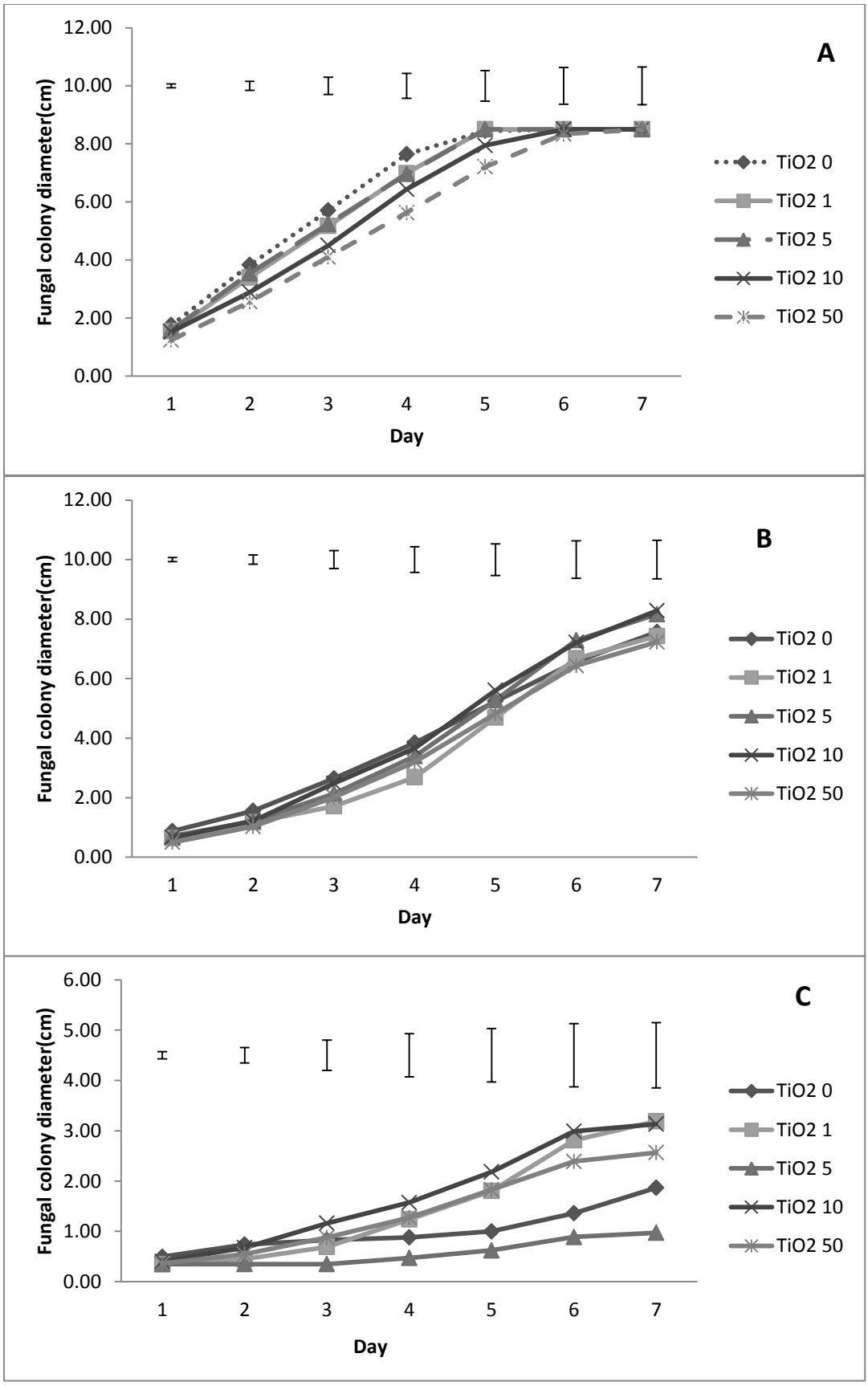


Fig.1 *Sclerotinia homoeocarpa* growth for non-treated UVB exposure control (plates kept in dark (0 mol $m^{-2}d^{-1}$) and two UVB exposures (1 (0.0918 mol $m^{-2}d^{-1}$) or 3 hr day^{-1}(0.2754 mol $m^{-2}d^{-1}$) treatments over time.

[1]Error bars represent Fisher's protected $LSD_{0.05}$.

Fig.2 Effect of TiO_2 concentration (0, 1, 5, 10, 50 mg ml^{-1}) on *in vitro Sclerotinia homoeocarpa* growth with three UVB radiation intensities: A. dark (0 mol $m^{-2}d^{-1}$); B. 1hr day^{-1}(0.0918 mol $m^{-2}d^{-1}$); C. 3hr day^{-1}(0.2754 mol $m^{-2}d^{-1}$).

[1]Error bars represent Fisher's protected $LSD_{0.05}$.


A
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10.00
8.00
6.00
4.00
2.00
0.00
Fungal colony diameter(cm)
1
2
3
4
5
6
7
Day
TiO2 0
TiO2 1
TiO2 5
TiO2 10
TiO2 50
B
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Fungal colony diameter(cm)
1
2
3
4
5
6
7
Day
TiO2 0
TiO2 1
TiO2 5
TiO2 10
TiO2 50
C
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Fungal colony diameter(cm)
1
2
3
4
5
6
7
Day
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TiO2 10
TiO2 50

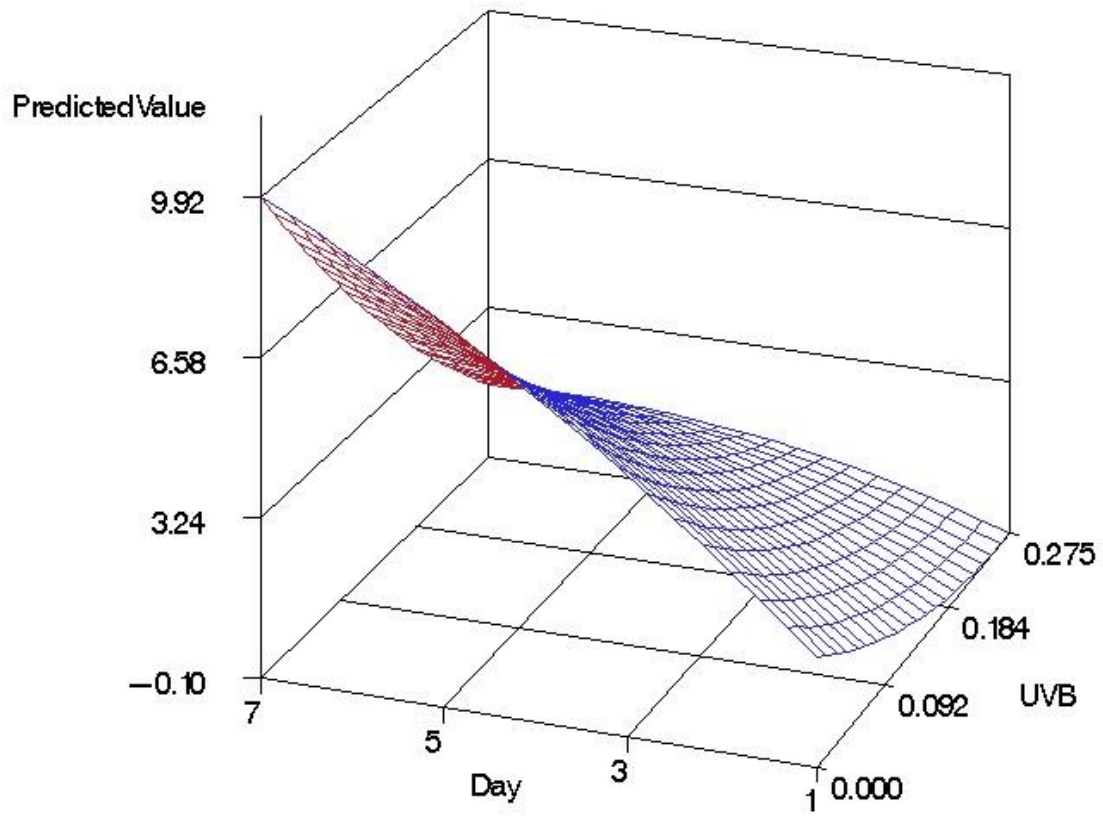


Fig.3 Response surface regression of the effect of day after inoculation and UVB radiation (mol $m^{-2}d^{-1}$) on *in vitro Sclerotinia homoeocarpa* colony diameter (R^2=0.9045).

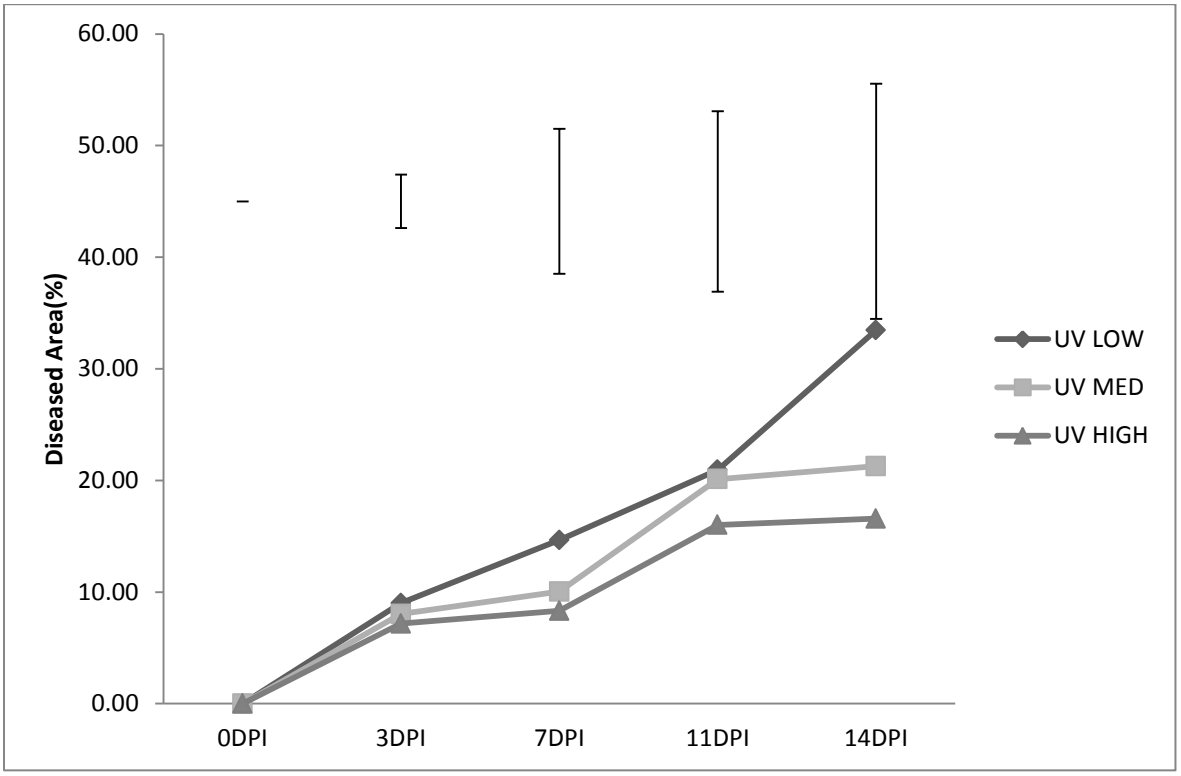
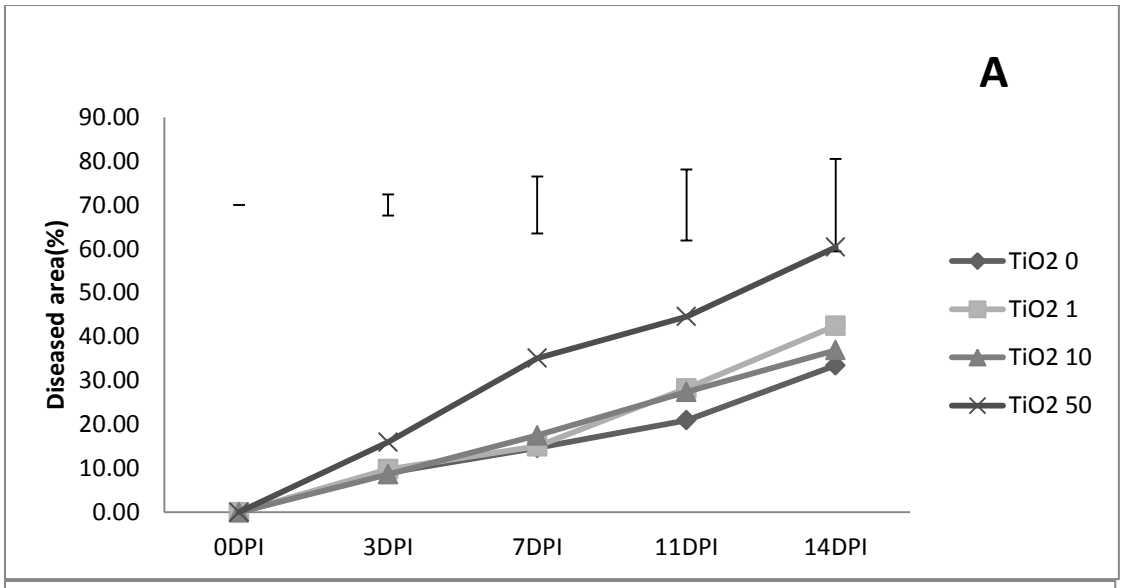


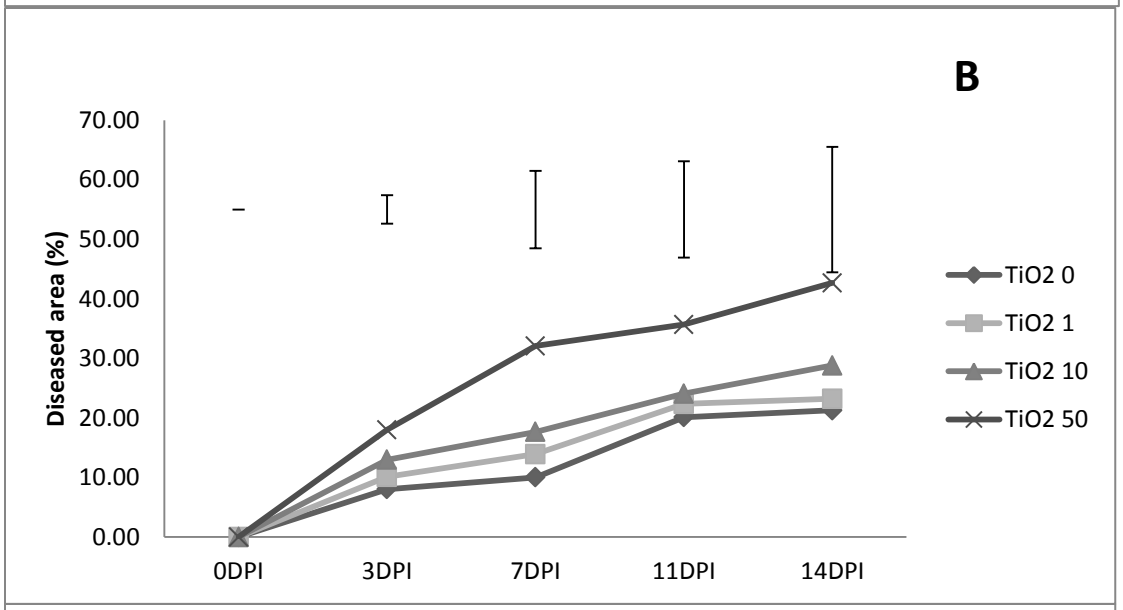
Fig.4 Effect of UVB exposure (Low (0.0454 mol $m^{-2}d^{-1}$), Medium (0.1064 mol $m^{-2}d^{-1}$), and High (0.5444 mol $m^{-2}d^{-1}$) on dollar spot disease development in pots of creeping bentgrass.

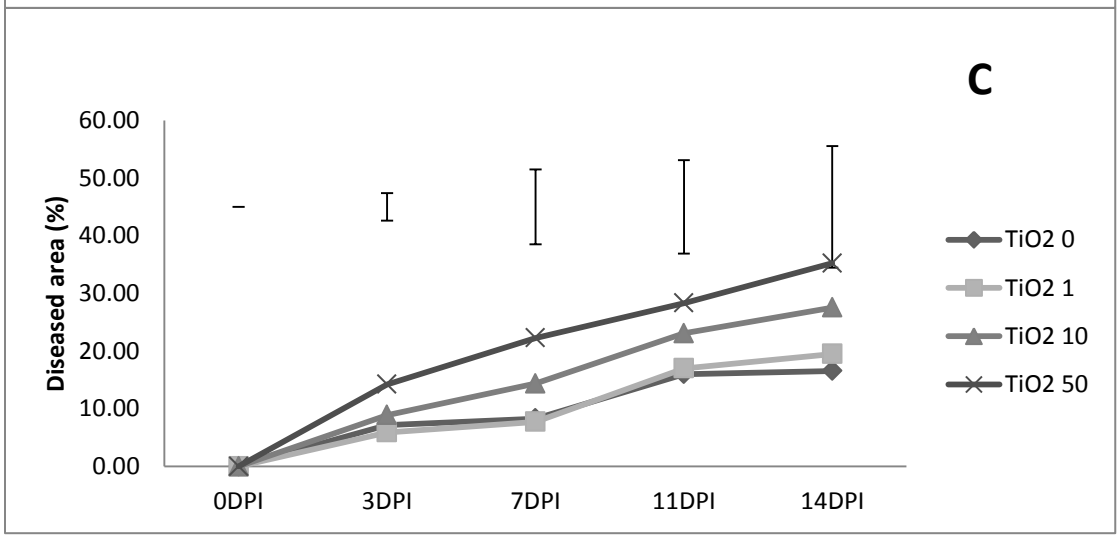
[1]**Error bars represent Fishers protected $LSD_{0.05}$.**

Fig.5 Effect of TiO_2 concentration on dollar spot disease development in pots of creeping bentgrass under three UVB exposures: A. Low (0.0454 mol $m^{-2}d^{-1}$); B. Medium (0.1064 mol $m^{-2}d^{-1}$); C. High (0.5444 mol $m^{-2}d^{-1}$).

[1]**Error bars represent Fishers protected $LSD_{0.05}$.**


A
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Diseased area(%)
0DPI
3DPI
7DPI
11DPI
14DPI
TiO2 0
TiO2 1
TiO2 10
TiO2 50


B
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Diseased area (%)
0DPI
3DPI
7DPI
11DPI
14DPI
TiO2 0
TiO2 1
TiO2 10
TiO2 50


C
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Diseased area (%)
0DPI
3DPI
7DPI
11DPI
14DPI
TiO2 0
TiO2 1
TiO2 10
TiO2 50

VITA

Chun Hu was born in Jiangyou, Sichuan, China in 1987. She received her Bachelor of Science degree from the Sichuan Agriculture University and Michigan State University. In the fall of 2010 she started her graduate studies in Plant Sciences and worked as a graduate research assistant in Plant Science Department at the University of Tennessee at Knoxville under the direction of Dr. Brandon Horvath. Upon graduation Chun plans on move to Ohio with her husband and pursue a Master's degree in statistics.